

CONFORMATION STUDIES OF PECTIC ACID*

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PALMER AND HARTZOG¹ from X-ray diffraction studies reported a value of 13.1 Å for the fibre repeat of sodium pectate. Though values of 12 to 13.1 Å were observed for the fibre repeat in the X-ray photographs of poorly oriented samples of pectic acid, Palmer² suggested the same value as that of sodium pectate. From the occurrence of third and sixth meridional lines in the X-ray diffraction pattern, a three-fold screw symmetry was suggested. From model building, Stone³ suggested 0-3'→0-5 type of hydrogen bond between contiguous residues. When the present work was completed Rees and Wight⁴, following the hardsphere approach, proposed a probable conformation for pectic acid assuming a value of 13 Å, the approximate value for the fibre repeat suggested by Palmer². However these authors have not examined the possibility of structures with three-fold screw symmetry corresponding to the fibre repeats in the range 12–13 Å. As stated in the earlier paper⁵ the studies based on the contact criteria are of limited use, since they fail to convey the relative stabilities of the various possible conformations. Hence in order to determine the energetically preferred conformations the potential energy has been computed for an isolated pectic acid chain by taking into account the non-bonded, torsional and the hydrogen bond energies.

Recently Rees and Wight have shown from n.m.r. studies⁴ that α-D-galacturonic acid residues have the C1(D) conformation⁶. Energy calculations⁷ have also indicated that the C1(D) conformation of galacturonic acid has about 1.7 kcal. mole⁻¹ lower energy than the 1C(D) form. Hence the various atoms in the galacturonic residues (Fig. 1) were fixed in C1(D) conformation by using the bond lengths and angles reported earlier⁸. The calculations were performed for two different bridge angles $\tau = 116^\circ$ and 119° . But the results obtained with $\tau = 116^\circ$ alone are reported here since the results obtained with $\tau = 119^\circ$ did not differ significantly. Section of a pectic acid containing a pair

of sugar residues is shown in Fig. 1. The hydrogen atoms attached to the ring carbons are not shown. Hydroxyl hydrogens and the atoms attached to C-6 atom were not fixed since their positions are expected to vary with the environment of the residues. The rotational angles ϕ and ψ specify (Fig. 1) the relative orientations of the galacturonic residues. The definition of the initial conformation $(\phi, \psi) = (0^\circ, 0^\circ)$ is the same as described earlier⁵.

For the computation of the disaccharide and helix energy, the expression

$$V = V_{nb} + V_{hb} + V_t \quad (1)$$

has been used, where V_{nb} is the non-bonded interaction energy term, V_{hb} , the hydrogen bond energy term and V_t , the torsional energy term. The hydrogen bond search was limited between the oxygen atoms 0-2, 0-3, 0-4, 0-5 of the unprimed unit with the oxygen atoms 0-1', 0-2', 0-3', 0-5' of the primed unit (Fig. 1). The form of the energy functions and constants used are the same as described earlier⁵. In computing the helix energy all non-bonded interactions of a residue over one turn of the helix were considered.

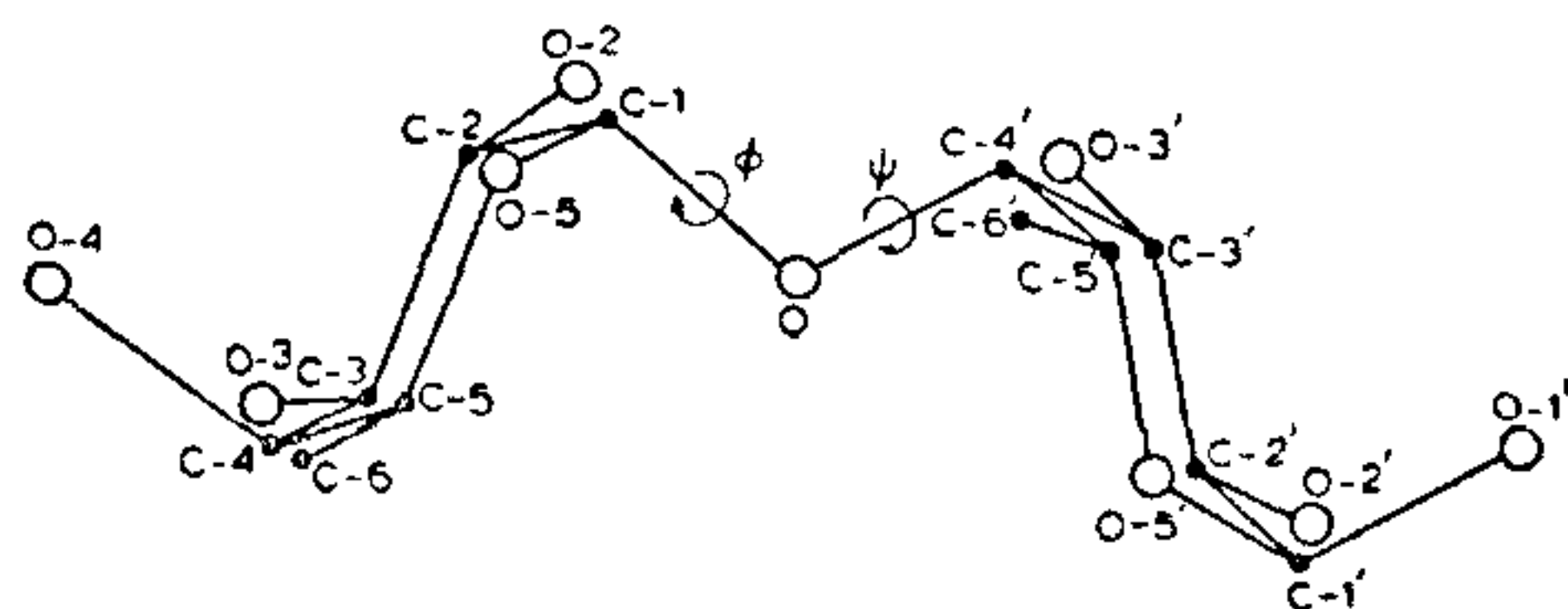


FIG. 1. Schematic diagram of a pair of α-D-galacturonic acid residues joined through (1 → 4) linkage. The directions of rotations of ϕ and ψ are marked.

The helical parameters, n , the number of residues per turn and h , the unit translation height along the helix axis, were calculated for the complete range of ϕ and ψ from -180° to $+180^\circ$ at intervals of 10° . The sense of the helix is represented by giving sign to n .

In Fig. 2 steric map (allowed region) obtained using the contact distance criteria⁹ has been

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superimposed on the energy contours for comparison. The small allowed region indicates that the two α -D-galacturonic acid residues can be joined through (1 \rightarrow 4) linkage without steric strain. The allowed region constitutes only about 2% of the total area on the (ϕ , ψ) map indicating that the freedom of rotation of the monomer units are highly restricted. Figure 3 illustrates the energy contours for helical pectic acid chain. The close similarity in the energy contours (Figs. 2 and 3)

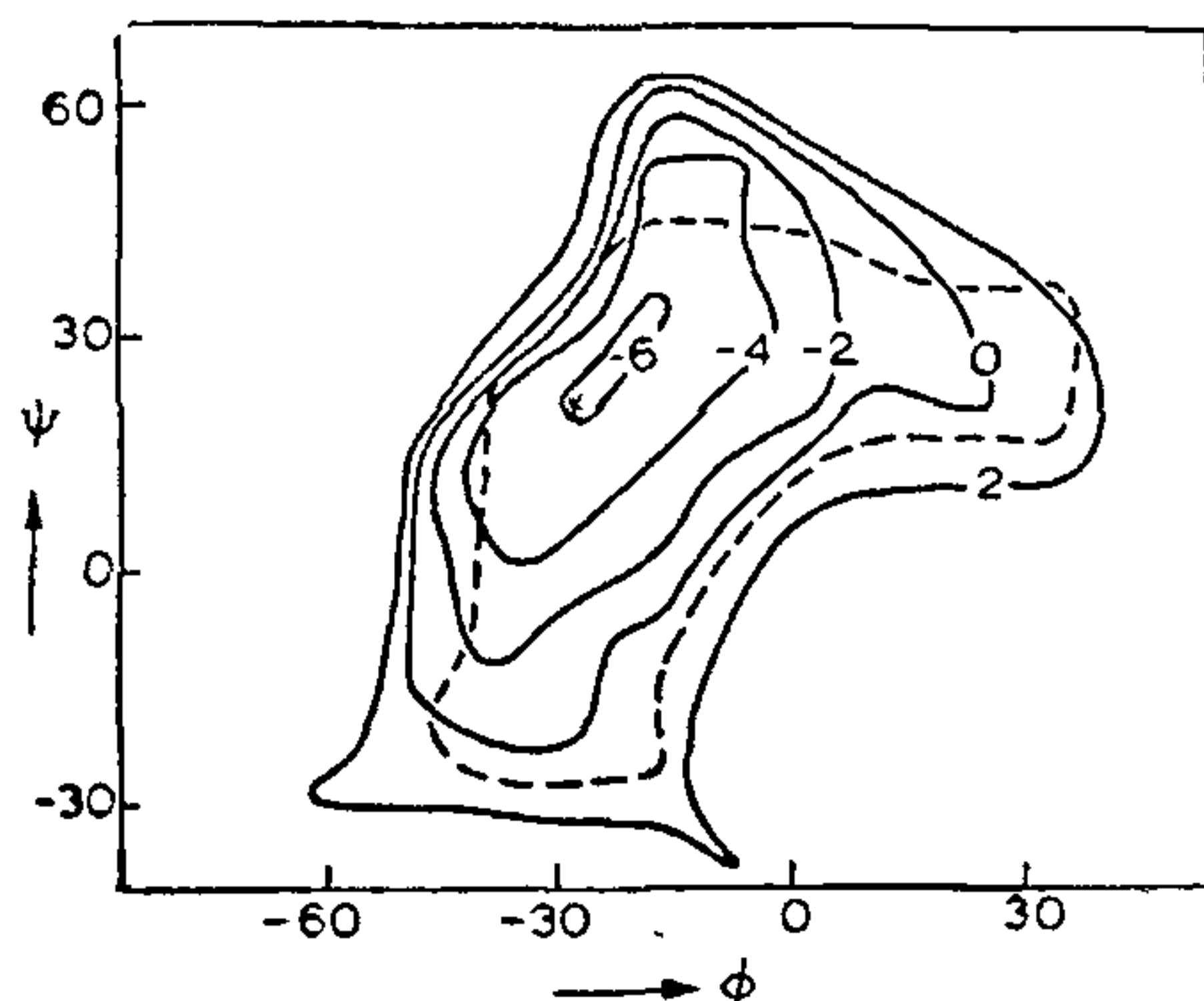


FIG. 2. Energy contours (—) obtained for a pair of α -D-galacturonic acid residues joined through (1 \rightarrow 4) linkage for $\tau=116^\circ$. Numbers on the contours indicate the energy values in kcal/mole. X-position of minimum energy Steric map (— · —) is indicated.

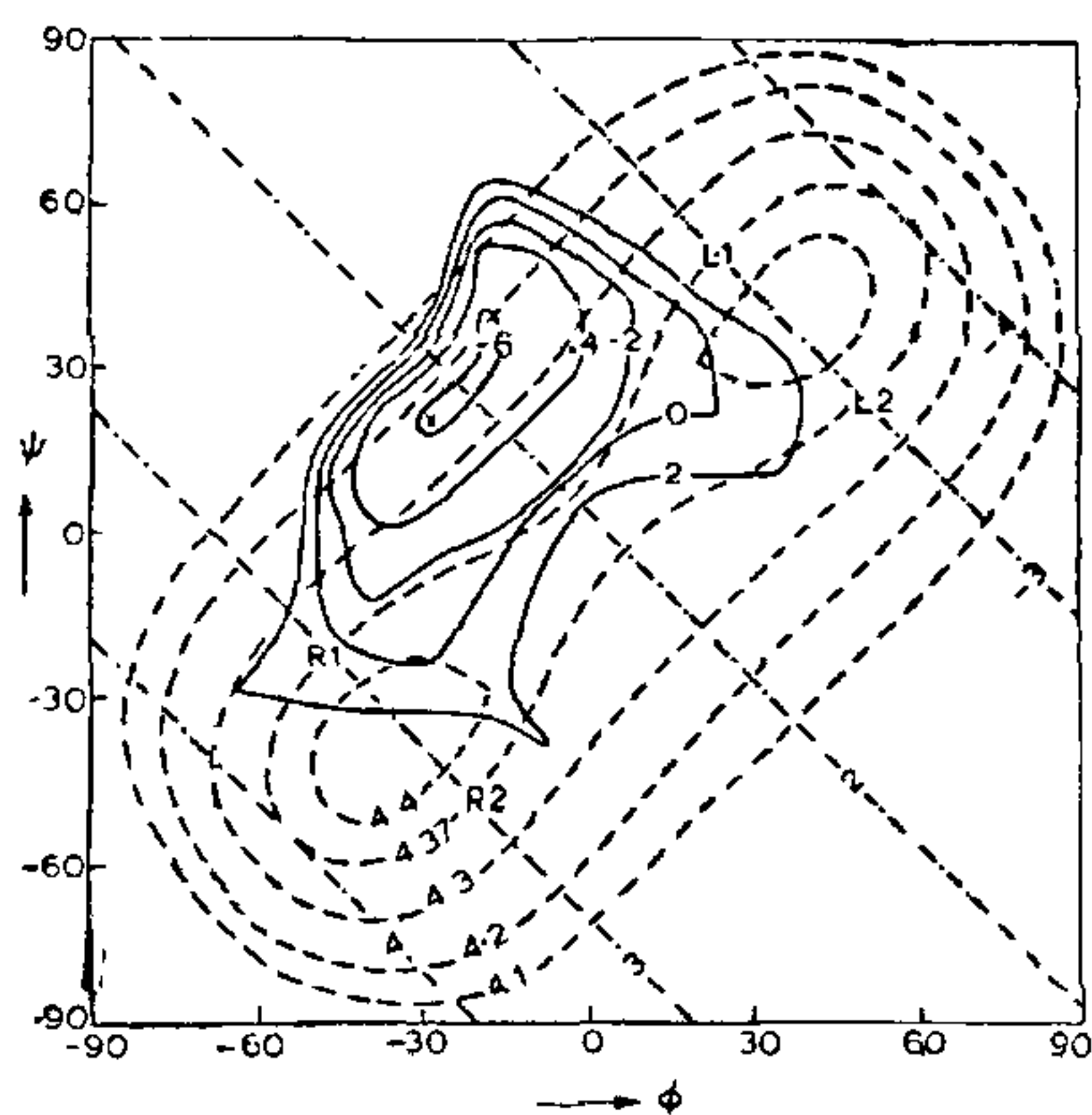


FIG. 3. Energy contours (—) in kcal/mole per residue for a pectic acid chain along with the curves of iso n (— · —) and iso h (— · —).

R1, R2: Positions of occurrence of right-handed helices with $n=3$, $h=4.37$ Å.

L1, L2: Position of occurrence of left-handed helices with $n=-3$, $h=4.37$ Å.

X: Position of minimum energy.

indicates that no additional restrictions are imposed on the freedom of monosaccharide residues as one passes from disaccharide-like to polysaccharide-like structures.

It is seen from Fig. 3 that low values of n between 2 and ± 3 and very high values of h in the range 4.1 to 4.4 Å close to the intra-residue distance of 4.4 Å are preferred suggesting that pectic acid chain favour extended or helical conformations with low values of n .

Since Palmer² preferred a helical structure with a pitch of 13.1 Å involving three sugar residues for pectic acid, the points of intersection of the curves $n = \pm 3$ and $h = 4.37$ Å are marked in the diagram (Fig. 3). Both right- and left-handed helices occur at two places with the above n and h values, and of these, the conformation R1 (-52° , -17°) has the minimum energy. The energy difference between R1 and the next nearest conformation namely R2 (-16° , -53°) is about 15 kcal per mole per residue. This clearly shows that the right-handed helical conformation R1 (-52° , -17°) is preferred energetically. From hard-sphere model Rees and Wight⁴ also preferred the right-handed helical sense for pectic acid chain and is also seen from Fig. 3 that the conformations having $n=3$ and h less than 4.3 Å are energetically unfavourable indicating that the value of 13 Å or more suggested by Palmer² for the fibre repeat of pectic acid, seems to be reasonable. This value might vary between 12.9 Å and 13.2 Å.

Even though the hydrogen bond search has revealed the possibility of 0-3'---0-5 hydrogen bond between contiguous residues in the allowed region, such hydrogen bonds are not possible in this particular conformation (-52° , -17°). Hence it seems that 0-3'---0-5 type of hydrogen bond proposed by Stone³ may not be possible in Palmer's structure with this geometry for the pyranose ring. The conformation R1 (-52° , -17°) occurs far away from the minimum energy position (Fig. 3). However it occurs very near the van der Waals minimum suggesting that this conformation is stabilized by van der Waals forces.

It is seen from Fig. 3 that the conformation R1 (-52° , -17°) has about 7 kcal/mole residue, higher energy than the minimum energy conformation. The large difference in the energy partly arises due to the possibility of 0-3'---0-5 type of hydrogen bond in the latter case. Our studies also reveal that the possibility of hydrogen bonds involving 0-6 atom with 0-2 of the neighbouring residue in agreement with the studies of Rees and Wight⁴. Such a hydrogen bond is also favoured

in the minimum energy conformation and also in conformations with parameters ($n = 2$, $h = 4.2$ to 4.3 Å). It is also seen from Fig. 3 that conformations with $n = 2$ and $h = 4.2$ to 4.3 Å, occur very close to the minimum energy. However, from the present calculations based on isolated chain it is not quite clear why the polygalacturonic acid chain prefers a three-fold screw axis rather than a two-fold as in cellulose¹⁰⁻¹². Probably for pectic acid the intermolecular forces play a major role in stabilizing helical conformations with three fold axis in solid state.

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1. Palmer, K. J. and Hartzog, M. B., *J. Amer. Chem. Soc.*, 1945, 67, 2122.
2. —, In: *High Polymer Physics, Symposium*, Ed. by H. A. Robinson, Page 42, Ramsen Press Division, Brooklyn, N.Y.
3. Stone, A. L., In: *Biological Macromolecules*, Vol. 2, Ed. 2, Ed. by S. N. Timasheff and G. D. Dasman, Marcel Dekker, N.Y., Chapter 6, 1969.
4. Rees, D. A. and Wight, A. W., *J. Chem. Soc.*, 1971, 7, 1366.
5. Sathyanarayana, B. K. and Rao, V. S. R., *Biopolymers*, 1971, 10, 1605.
6. Eliel, E. L., Allinger, N. L., Angyal, S. J. and Morrison, G. A., In: *Conformational Analysis*, Interscience (John Wiley), Chapter 6, 1965.
7. Sathyanarayana, B. K. and Rao, V. S. R., unpublished data.
8. Sundararajan, P. R. and Rao, V. S. R., *Tetrahedron*, 1968, 24, 289.
9. Ramachandran, G. N. and Sasisekharan, V., *Adv. Prot. Chem.*, 1968, 23, 283.
10. Mayer, K. H. and Misch, L., *Helv. Chim. Acta*, 1937, 20, 232.
11. Wellard, H. J., *J. Polymer Sci.*, 1954, 13, 471.
12. Marchessault, R. H. and Sarko, A., *Adv. Carbohydr. Chem.*, 1967, 22, 421.

INFRARED ABSORPTION SPECTRA OF META- AND PARA-METHYLANISOLES

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ABSTRACT

The infrared absorption spectra of meta- and para-methylanisoles have been recorded in the region $350\text{--}4000\text{ cm}^{-1}$ in liquid phase and the vibrational analyses have been performed. The observed fundamental frequencies have been assigned to various modes of vibrations with the assignments proposed for anisole and some of its derivatives and for other disubstituted benzenes.

INTRODUCTION

VIBRATIONAL analysis of the infrared and Raman spectra of anisole and its mono halogen substituted derivatives have been carried out by Owen and Hester¹, to investigate possible trends in the vibrational patterns of disubstituted benzenes. The assignments of the observed frequencies in substituted anisoles become somewhat more difficult because of the superposition of, perhaps, several vibrations due to the phenyl ring and those due to the substituents. However, it is possible to assign the substituent vibrational modes from a comparison with assignments of similar substituents in simpler molecules. Josien and coworkers² working on a number of substances containing CH_3 group assigned the frequencies in 2900 cm^{-1} region to methyl group vibrations. They further observed that the methyl group vibrations are very slightly affected by the nature or position

of other substituents. In the present study the infrared spectra of meta- and para-methylanisoles have been investigated in liquid phase and an attempt has been made to assign the observed frequencies to various modes of vibrations.

EXPERIMENTAL

The chemicals used in the present investigations were manufactured by Fluka AG (Switzerland), and were of pure quality. These are liquids at room temperature.

The infrared absorption spectra of *m*- and *p*-methylanisoles were recorded in the region $650\text{--}4000\text{ cm}^{-1}$ in liquid phase. In each case a small amount of the sample was enclosed between two NaCl windows and was pressed without using any spacer and the absorption path length thus available was found sufficient. In the region $350\text{--}650\text{ cm}^{-1}$, the spectra of the pure liquids (meta- and para-isomers) were recorded by using a KBr cell of